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Hypernatremia deepens the demarcating borderline of leukocytic infiltration in the burn wound

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The influence of hypernatremia on progressive burn wound necrosis was evaluated in a second-degree burn model. The depth of the burned tissue sloughed off was measured in a comparative study in the rat (contact burn injury with a metal plate, 70°C for 5 s). Rats in the hypernatremic group were treated with an intraperitoneal fluid injection of hypernatremic saline (10 ml, 850 mEq/l). Control rats were injected with hyponatremic saline (10 ml, 100 mEq/l). On the fourth postburn day specimens were harvested and compared. The greatest depth of leukocytic infiltration (percent of total dermal thickness) was measured.

The average depths were significantly different. In zone I (from the normal skin edge to 5 mm inside the wound) the average depth in the hypernatremic group was 38.0±9.3 percent of the dermal depth and in the control group 9.5±1.2 percent. In zone II (from 5 to 10 mm inside the wound) the hypernatremic group sloughed off at 64.0±11.8 percent and the control group at 12.5±2.1 percent. The hypernatremic group showed a deeper demarcating borderline of leukocytic infiltration than the control rats. The wound depth progression may be caused by an osmotic injury. © *1997 Elsevier Science Ltd for ISBI. All rights reserved.*

Key words: Burns, burn wounds, neutrophils, osmolality, hypernatremia.

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Introduction

Clinicians who care for burn patients must be alert to progressive burn wound necrosis. In this phenomenon the coagulation zone of the burn wound increases in depth several days after the burn injury[1–4]. A superficial wound may change to a deep wound. Whether the coagulation zone involves the deep part of the dermis or not is very important. The outcome of therapy depends largely on the hair follicles as a source of skin epithelium regeneration. If these structures are well preserved, wounds may heal spontaneously in 2 weeks. If not, skin grafting may be required. Burn depth progression is an important turning point in the course of therapy. Prevention of such progression is of great importance and the topic of considerable recent research[5].

The causes of this phenomenon have been attributed to an insufficient blood supply[1] or dehydration[4] at the stasis zone just below the zone of coagulation. Our observations of many burned patients suggest that there may be other explanations for the natural history of this progression. To investigate why and how this pathological change occurs, we considered complications occurring after the acute phase (involving the shock and refilling phases). One possible factor is the close relationship between hypernatremia and wound depth progression (personal observations). This suggests that hypernatremia, an osmotic injury, is a promoting factor involved in wound depth progression or cell death at the zone of stasis which has suffered a heat injury as an initiating factors[6]. This hypothesis led us to investigate the pathological features of the borderline between the zone of coagulation and stasis in the burned rats treated with hypo- and hypernatremic saline. Leukocytes infiltrate the burn wound to prevent bacterial infection and to demarcate the damaged tissue which will be sloughed off[7]. Observation of the depth of the demarcating borderline of leukocytic infiltration suggests that hypernatremia may be regarded as a cause of progressive burn wound necrosis.

Material and methods

An experimental skin burn model as described below was used in this study. Animals were treated in strict accordance with the Guidelines for Animal Experimentation decided by the Ethical Committee of Saitama Medical School. Adult male Wistar rats (280–300 g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The skin on the back was shaved and thoroughly depilated with a cream. Thermal injury, the second-degree burn wound, was made by a contact with a

metal plate (70°C, 5 s) on the dorsal skin[8]. Two round wounds (2 cm in diameter) were made on both left and right flanks. Blisters were left intact. Intraperitoneal fluid administration was performed as described below. Rats in the hypernatremic group ($N = 5$) were given 10 ml of hypernatremic saline (850 mEq/l of sodium concentration). Rats in the control group ($N = 5$) were given hyponatremic saline (10 ml, 100 mEq/l) to prevent hypernatremia under the condition of increasing evaporative water loss from the wound surface. This hyponatremic solution is isotonic with the glucose solution in many of the fluid formulae which are used clinically. After treatment food and water were given freely.

On the fourth postburn day two specimens of the burned skin, including subcutaneous muscle and the adjacent normal skin, were harvested from each rat. Routine hematoxylin and eosin staining was performed. The depth of the demarcating borderline of leukocytic infiltration in the dermis was measured. In our study we selected the fourth postburn day because after the fifth day the zone over the infiltrating borderline begins to shrink and is sloughed off (*Figure 1*). Sampling on the fourth day avoided measurement errors caused by the shrinking and sloughing. The deepest points in Zone I (from the normal skin edge to 5 mm inside the wound) and in Zone II (from 5 to 10 mm inside) were measured and

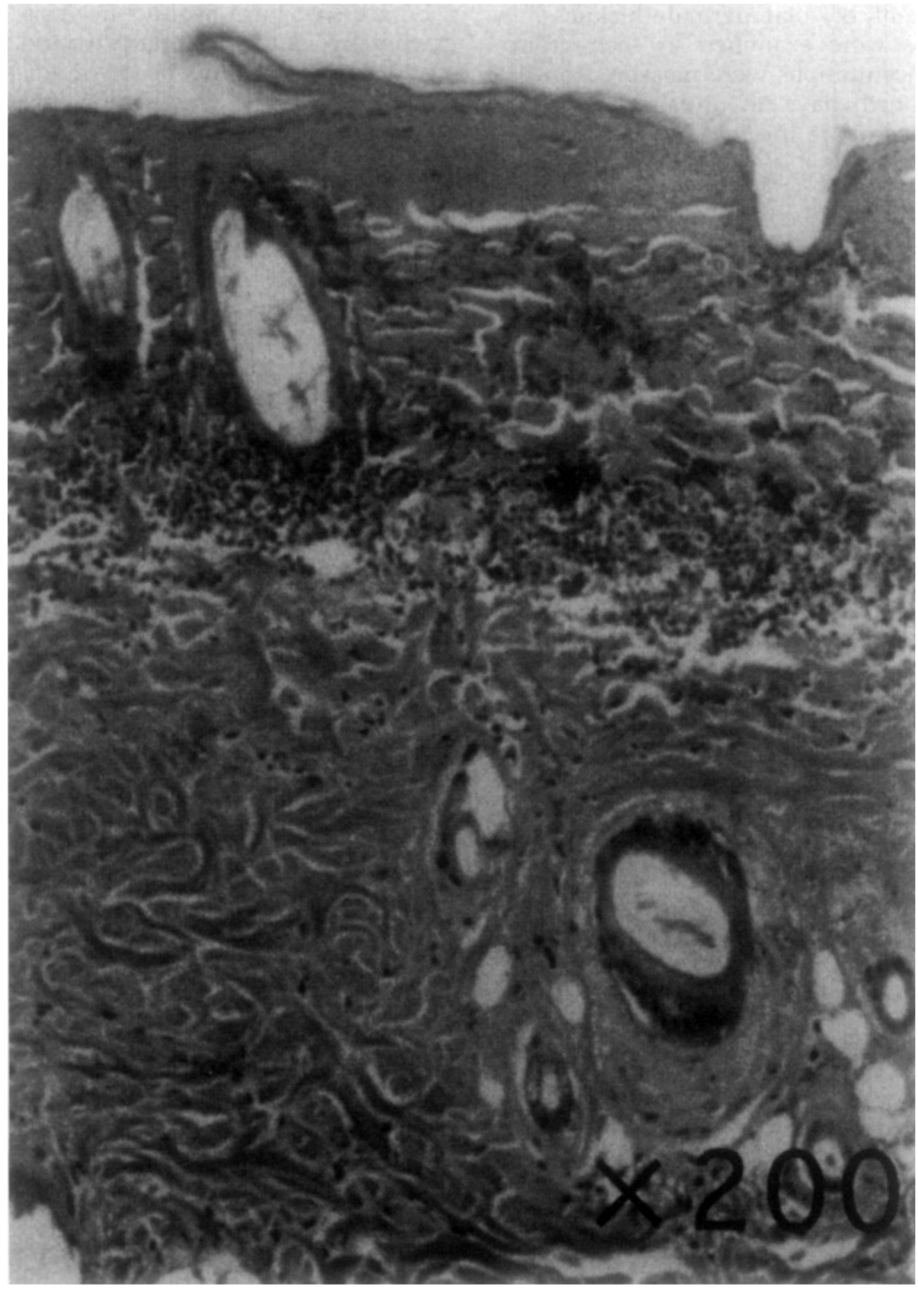

Figure 1. Demarcation of the burned tissue to be sloughed off (hematoxylin and eosin stain). After the fifth postburn day the upper portion of the dermis above the demarcating borderline of leukocytes begins to be sloughed off by the digestive activity of neutrophils.

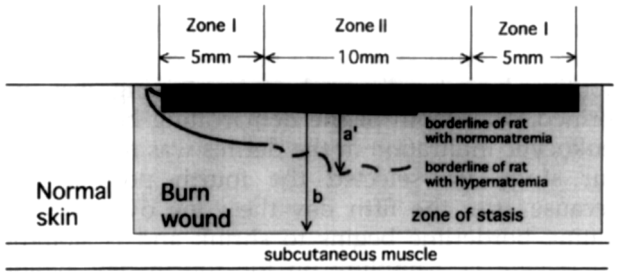

Figure 2. Scheme of the burn wound. The demarcating borderline in a rat with hypernatremia is deeper than that of a rat with normonatremia. The depth was measured as per cent total dermal thickness (a or $a' \div b \times 100$).

recorded photographically (*Figure 2*). The depth was expressed as percent of total dermal thickness. In total 10 specimens were examined in each group. Serum sodium concentrations were measured on the second and the fourth day. An unpaired Student's *t*-test was used to compare the measurements in the two groups.

Results

The experimental results are summarized in *Tables I* and *II*. The average depths of the demarcating borderline of leukocytic infiltration at which sloughing off occurred were significantly different. In Zone I the average depth in the hypernatremic group was 38.0 ± 9.3 percent of dermal depth and in the control group 9.5 ± 1.2 percent. In Zone II the hypernatremic group was 64.0 ± 11.8 percent of dermal depth and in the control group 12.5 ± 2.1 percent. Serum sodium concentrations differed statistically on the second day but there was no significant difference on the fourth day. The hypernatremic group formed deeper demarcating borderlines of leukocytic infiltration than those of control rats. This infiltrating borderline was clearer in Zone I and showed gradual deepening from the normal skin edge to the inside of the wound. In Zone II the borderline became deeper and disrupted (*Figure 2*).

Specimens from the hypernatremic and control groups are shown in *Figures 3* and *4*. There is a distinct difference in the depth of leukocytic infiltration. Moreover in the specimen with hypernatremia disappearance of the hair follicles, apparently through an apoptotic process, was observed[6].

Table I. Comparison of the depth of the demarcating borderline for the hypernatremic and hyponatremic solution (control) groups (each $N = 10$)

	Zone I (percent)	*Zone II (percent)*
Hypernatremic group	38.0 ± 9.3^a	64.0 ± 11.8^a
Control group	9.5 ± 1.2	12.5 ± 2.1

Values are means ± SEM, representing percent total dermal thickness of the demarcating borderline of leukocytic infiltration in the dermis.
Zone I is the area from the normal skin edge to 5 mm inside the wound and Zone II is from 5 to 10 mm inside.
$^aP < 0.01$ for the comparison with control group.

Table II. Serum sodium concentration of the hypernatremic and the control groups (each $N = 5$)

	Serum sodium concentration (mEq/l)	
	2nd day	*4th day*
Hypernatremic group	162.4 ± 5.2^a	143.2 ± 3.8
Control group	142.0 ± 3.7	141.5 ± 4.0

Values are means ± SEM.
$^aP < 0.01$ for the comparison with control group.

Discussion

Progressive burn wound necrosis is a phenomenon whereby a superficial burn wound increases in depth and leads to a deeper wound than the primary wound. Such a progression is an important and unsolved problem for the clinicians who care for more severely burned patients because it has a significant influence on the outcome of burn therapy. A superficial dermal burn (SDB) heals spontaneously within almost 2 weeks. In such a wound the skin, hair cells, etc., are well preserved and function as a source of skin regeneration. A deep dermal burn (DDB) often does not heal successfully because many of these structures are lost. In DDB hypertrophic scarring often occurs and skin graft operations and long hospital stays are frequently needed. Therefore, progressive burn wound necrosis, especially from SDB to DDB, is a critical problem for patients and clinicians. The causes of the phenomenon are largely unknown and require future research[5].

Some reports suggest an insufficient blood supply and dehydration as causes of progressive necrosis at the zone of stasis. The stasis zone just below the zone of coagulation is an unstable site during 7 days postburn and may, or may not be preserved[9,10]. Prolonged interference with the blood supply is thought to increase the depth of necrotic tissue of this site[1]. Another possibility is that dehydration of the wound causes cell death and leads to an increase in the depth of necrotic tissue[4].

In our clinical experience we have used a uniform method, Vaseline gauze with some antibiotics, as a local treatment for SDB. However, the primary predictions of wound fat were unreliable in many patients which is in line with previous reports[11–13]. Consideration of how and why this progression occurs led us reflect on complications occurring after the acute phase and also any relationship between these and fluid status. Subsequently we observed a close relationship between hypernatremia and progressive wound necrosis[6]. This relationship suggested that hypernatremia may promote progressive wound necrosis.

The average depth of the demarcating borderline of leukocytic infiltration in the hypernatremic group was deeper than that of the control group. This means that hypernatremia causes a greater thickness of tissue to be sloughed off. Two mechanisms by

which leukocytes decide where the demarcating borderline should be drawn can be hypothesized. The first is selective migration[14]. The cells and extracellular matrix at the superficial layer are more severely damaged by heat and osmotic injury. These may not be repairable by their own cell repairing mechanism. It is possible that the cells produce signs of 'critical illness', specific antigens on the cell surface, cytokines as paracrinal signals, and so forth, showing that they can no longer survive[15,16]. Recognizing and judging this information about damaged cells, leukocytes may gather at the borderline to separate the unsalvageable tissue. The second hypothesis is suppressive immobility. The chemotactic function of the leukocytes is suppressed under the various environments related to the burn injury[17] and under hyperosmotic, especially hypernatremic, conditions[18]. This is probably due to the exhaustive consumption of intracellular adenosine triphosphate (ATP) by the Na^+–K^+ pump[19]. This pump is an ATPase and consumes more intracellular ATP under hypernatremic conditions in order to maintain the intracellular hyponatremic condition[20]. Therefore under hypernatremic conditions the leukocytic chemotactic function is suppressed and leukocyte mobility is inhibited at the limiting sodium concentration[18]. Leukocytes cease to migrate when the limiting point in the osmotic gradient of the dermis is

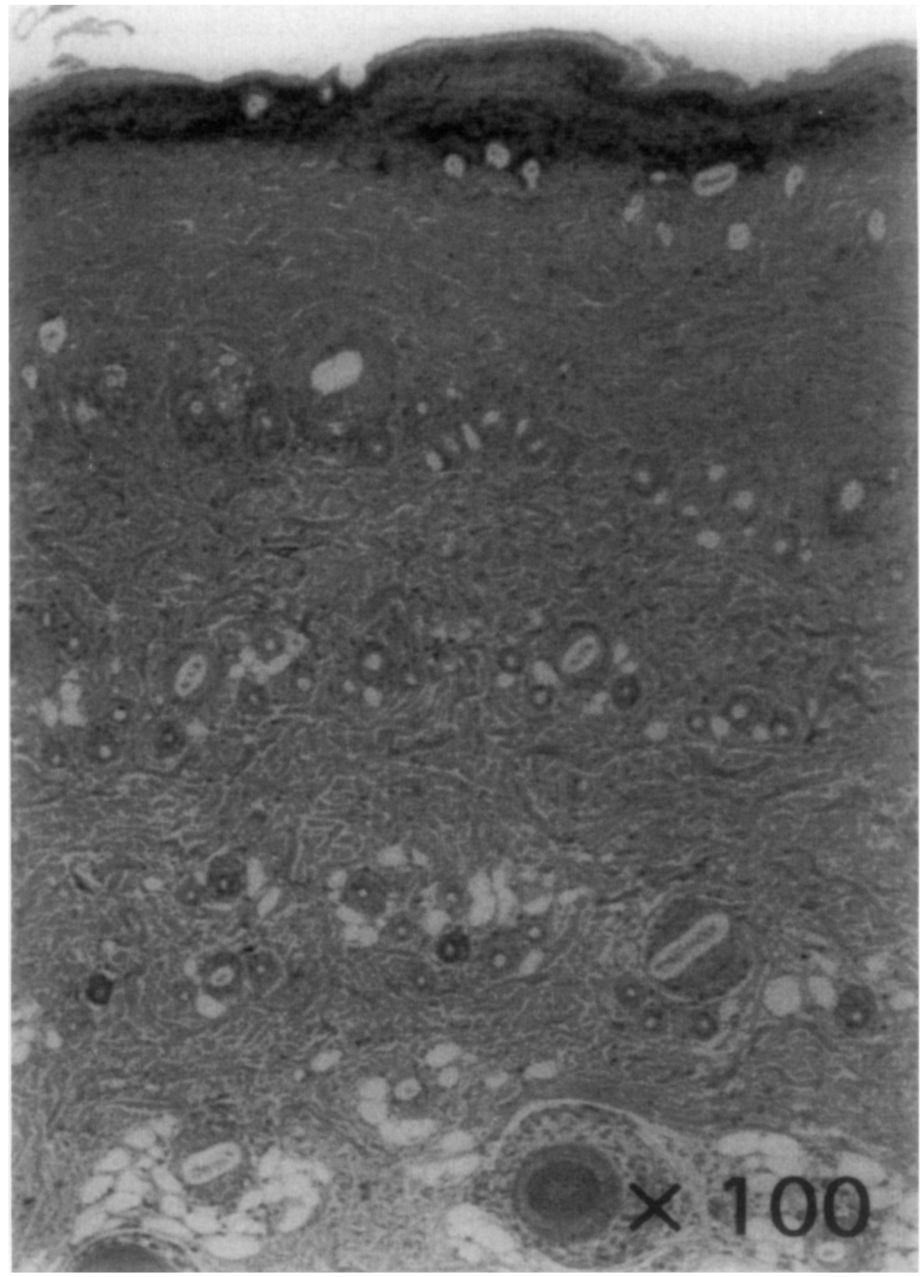

Figure 3. Leukocytic infiltration after treatment with hyponatremic saline on the fourth postburn day (hematoxylin and eosin stain). The demarcating borderline of leukocytes is limited near to the epidermis. Sebaceous glands are under this borderline and are well preserved.

reached. The highest point of this gradient will occur at the surface of the wound and the lowest point, similar to the serum sodium concentration, at the deep zone of the dermis where an almost normal blood circulation is maintained. Leukocytes will reach the point at which migration is inhibited and will begin to damage the surrounding tissue.

We consider that our results support this suppression theory, since there were many sporadic or clustered leukocytes through the coagulation zone which had previously been the zone of stasis. This suggests that active migration from the superficial layer (high osmotic concentration) back to the deep layer (normal osmotic concentration) is impossible. Involving the viable interstitial and migratory cells above, the large number of leukocytes digest the infiltrating tissue at the demarcating borderline and separate the upper portion of the dermis as a slough.

Free water loss may occur more easily than sodium loss because of the increasing evaporative water loss from the burn wound surface[21]. This means that hypernatremic conditions may occur in the zone of stasis as a result of an insufficient blood supply and dehydration. Serum hypernatremia will accelerate the hypernatremic tendency near the wound surface. From our study the fate of the

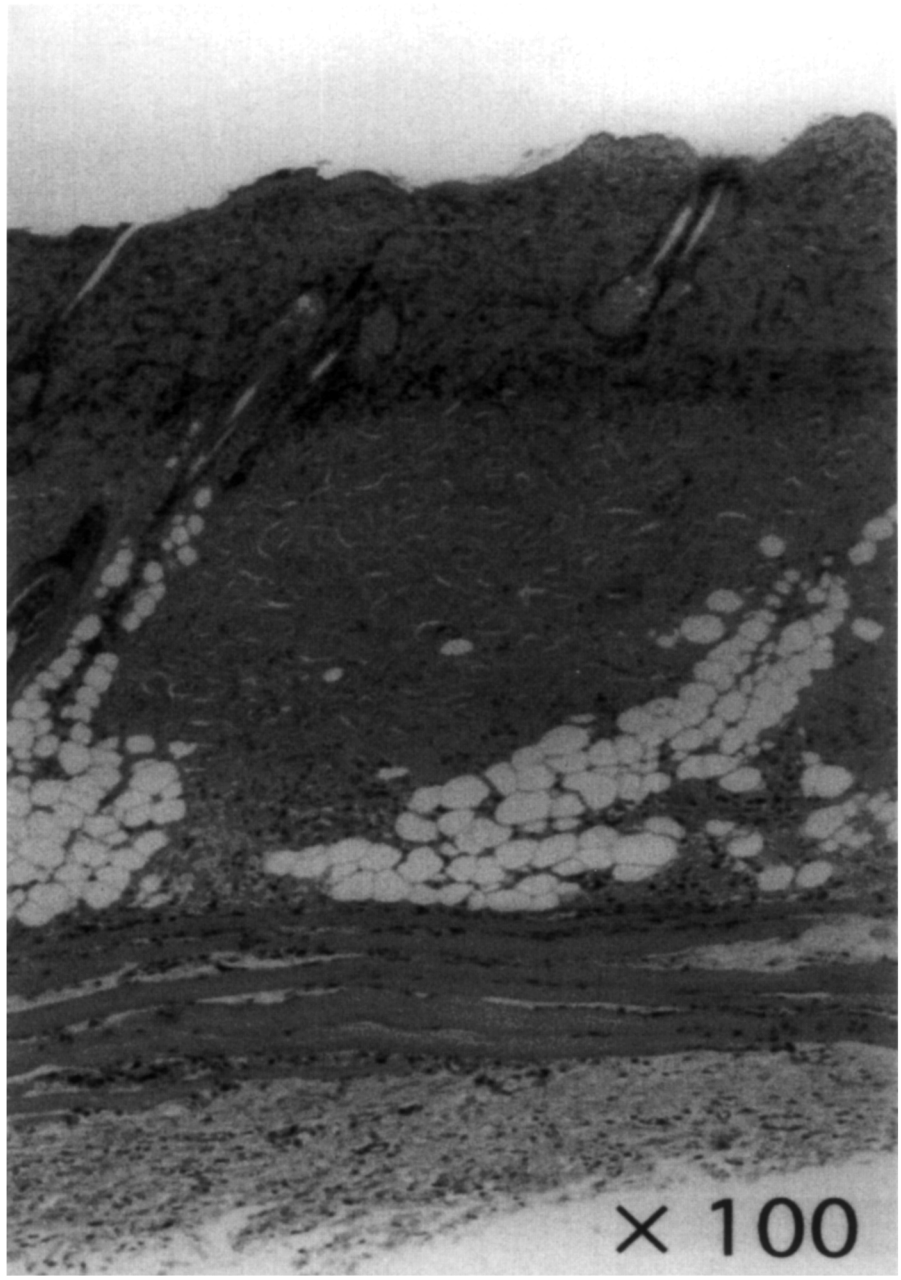

Figure 4. Leukocytic infiltration after treatment with hypernatremic solution on the fourth postburn day (hematoxylin and eosin stain). The demarcating borderline of leukocytes is deeper than that of a control rat. Sebaceous glands are above this borderline and are already necrotic. Hair follicles are under this line, but are lost by an apoptotic process due to an osmotic injury[6].

demarcating borderline may be strongly influenced by serum hypernatremia at the early stage of shock resuscitation. This means that fluid resuscitation may significantly and unexpectedly influence the burn wound.

These observations suggest the hyponatremic isotonic solutions as a resuscitation fluid administration may aid wound management. Many historic resuscitation formulae adopt such hyponatremic solutions because they include a 5 percent sugar solution to compensate for insensible water loss[22,23]. One method for preventing depth progression may be to supply sufficient free water which should be administered in the form of an isotonic solution of 5 percent sugar. This free water would dilute the hypernatremic or hyperosmotic sites in the burn wound by the providing an osmotic equilibrating force and alleviate the potential risk there. Other possible methods are, of course, to promote recovery of the microcirculation at the zone of stasis with some effective agents and to prevent dehydration by effective external dressing materials.

As described previously, in our clinical observation there is a close relationship between hypernatremia and progressive wound necrosis. The correction of the body fluid condition, especially serum sodium concentration and osmolality, during shock resuscitation is a precondition for the preservation of the primary wound depth. Furthermore we recognize the increased correlation of burn wound sepsis and resultant renal failure as one aspect of multiple organ failure (MOF) associated with hypernatremia. A recent report also suggests disadvantageous results of the use of hypertonic sodium solutions[24]. Hypernatremia has not been regarded as an important factor through the shock period, because its direct effects on several complications are not well enough investigated. Although the impairment effects on normal tissue may not be large, it is possible that osmotic injury as a further insult is a severe problem for heat damaged tissue and leads to irreversible damage or necrosis.

We would like to emphasize strongly the need to evaluate the influence of fluid resuscitation on wound depth progression and associating complications. As far as fluid resuscitation is concerned, every formula seems to be successful. Fluid therapy for cardiopulmonary resuscitation is not a serious problem in most recent intensive care regimes. The influence of fluid treatment for wound resuscitation on local tissue damage will be more important in future burn research.

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